

## REMARKS

After entry of this amendment, claims 1, 6, 26-28 and 31-32 are pending. Claims 3, 5, 7, and 30 are canceled without prejudice. Applicants reserve the right to prosecute subject matter of canceled claims in subsequent applications.

Claim 1 has been amended to recite a method for detecting whether a tyramine receptor binds to a G-protein and triggers an endogenous G-protein coupled signalling cascade of an erythroid cell comprising the steps of; transforming an erythroid cell according to claim 6 with a vector comprising a sequence which encodes a tyramine receptor under the control of a globin promoter, incubating the cell with a ligand capable of binding the tyramine receptor, measuring the cyclic AMP levels or free calcium ion concentration within the cell, comparing the cyclic AMP levels or the free calcium ion concentration with that of an untransformed erythroid cell, and detecting the tyramine receptor binding to a G-protein by observing a difference in the cyclic AMP levels or calcium ion concentrations measured between the transformed and untransformed erythroid cells. Support for these amendments is in the specification on page 4, lines 28-30, page 5, line 26-page 6, line 7, page 8, line 26-page 9, line 9, page 7, lines 18-19, Example 4, and Example 5.

Claim 6 has been amended to be independent and recite the erythroid cell line as deposited at the European Collection of Cell Cultures under Accession number 99012801. Claim 6 is no longer dependent upon canceled claim 30.

Claim 27 has been amended to recite the isolated erythroid cell according to claim 26 which has been further transformed such that it contains a globin promoter associated with a reporter cassette containing a  $\beta$ -galactosidase gene under the control of a response element susceptible to modulation by a signalling cascade of said cell. Support is in the specification on page 9, lines 23-25 and page 6, lines 8-10.

Claim 28 has been amended to recite the isolated erythroid cell according to claim 27 wherein said response element is the Locus control Region (LCR) enhancer, wherein said enhancer is at an optimal distance of said reporter cassette such that the expression of the  $\beta$ -

galactosidase gene is dependent on the concentration of a downstream component in the signalling cascade. Support is in the specification on page 9, lines 23-25.

Claim 31 has been amended to recite a method for detecting whether a tyramine receptor binds to a G-protein and triggers an endogenous signaling cascade of an erythroid cell comprising the steps of: providing the erythroid cell according to claim 27 or 28, incubating the cell in the presence of a ligand capable of binding the tyramine receptor, measuring the expression levels of the  $\beta$ -galactosidase gene, comparing the  $\beta$ -galactosidase expression levels with those of an untransformed erythroid cell, and detecting the binding of a tyramine receptor to a G-protein by observing a difference between the levels of  $\beta$ -galactosidase expression from transformed and untransformed erythroid cells. Support for these amendments is in the specification on page 4, lines 27-30, page 5, line 26-page 6, line 7, page 8, line 26-page 9, line 9, page 7, lines 18-19, and Example 6.

Claim 32 has been added to recite the method according to claim 1 wherein the calcium levels are measured by means of a fluorescent indicator. Support is in the specification on page 5, lines 26-30 and claim 10 as filed.

Claims 33 and 34 have been added to recite the method of claim 1 or claim 31, respectively, wherein the ligand is an agonist or antagonist and the incubation is performed either (I) in (a) the presence and (b) the absence of a potential agonist of the tyramine receptor and/or (II) in the presence of a known agonist and (a) the presence or (b) the absence of a potential antagonist of the tyramine receptor; and measuring and comparing the levels of cyclic AMP or calcium ion of (Ia) and (Ib) and/or (IIa) and (IIb). Support for these claims is in the specification on page 4, line 20-page 5, line 25 (see in particular page 4 lines 20-27).

No new matter has been added by these amendments.

### **Claim Objections**

Claim 5 was objected to for informalities. Claim 5 has been canceled making this objection moot.

### **Claim Rejection under 35 USC § 112, first paragraph**

A) Claims 5, 7 and 30 are rejected under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter which was not enabled by the specification.

Claims 5, 7 and 30 have been canceled without prejudice, making this rejection moot.

B) Claims 1 and 3 are rejected under 35 U.S.C. 112, second paragraph, as allegedly not being enabled for any erythroid cell, but are considered enabled for the deposited erythroid cell line.

Applicants respectfully disagree with this rejection. However, in order to advance prosecution of certain embodiments of the invention, claim 1 has been amended to recite a method using the deposited erythroid cell line, thereby overcoming this rejection. Claim 3 has been canceled without prejudice, therefore, making this rejection moot.

C) Claims 1, 3, 5, 7, 26-28 and 30-31 are rejected under 35 USC § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time of filing the application.

Applicants respectfully disagree with this rejection, however to advance prosecution of certain embodiments of the invention, claims 1, and 26-28 have been amended to use the deposited erythroid cell line of claim 6, thereby overcoming this rejection since the cell line was described and deposited. Claims 3, 5, 7, and 30 have been canceled without prejudice, thereby making this rejection moot.

### **Claim Rejection under 35 USC § 112, second paragraph**

A) Claims 1, 3, 5-7, and 30-31 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for using the term "interaction".

The term "interaction" has been deleted from the claims, thereby overcoming this rejection.

B) Claims 1, 3, and 31 are rejected as allegedly being incomplete for omitting the assay step.

Applicants respectfully disagree with this rejection, however, in order to advance prosecution of certain embodiments of the invention, claims 1 and 31 have been amended to recite the assay step, thereby overcoming this rejection. Claim 3 has been canceled, making this rejection moot.

C) Claims 1, 3, 5, 6, 7 and 30 are rejected under 35 USC § 112, second paragraph, as allegedly being incomplete for missing steps.

Applicants respectfully disagree with this rejection, however, in order to advance prosecution of certain embodiments, claim 1 has been amended to add steps of incubating with a ligand, comparing levels and detecting binding in order to clarify the claim. Claim 6 is now an independent claim to the deposited cell line.

Claims 3, 5, 7, and 30 have been canceled, thereby making this rejection moot.

D) Claims 1 and 3 are rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for lacking a step that does not clearly relate back to the preamble.

Claim 1 has been amended to recite a step of detecting which relates back to the preamble, thereby overcoming this rejection. Claim 3 has been canceled, making this rejection moot.

E) Claim 31 is rejected for the recitation of "a tyramine receptor" which allegedly lacks antecedent basis. Claims 26 and 31 have been amended to provide antecedent basis.

### Conclusion

In view of the above amendments, it is submitted that the application is now ready for allowance. If any additional information is needed, the Examiner is invited to call the undersigned attorney at (919) 765-5071.

Respectfully submitted,



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